Consensus Sequence

Symbol comparison table: blosum62.cmp CompCheck: 6430

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Name: hTRADEalpha_1     Len:   423  Check: 4050  Weight:  1.00
Name: hTRAIN_1          Len:   423  Check: 4128  Weight:  1.00
Name: hTRADEbeta_1      Len:   423  Check: 7937  Weight:  1.00
Name: hApo420pep        Len:   423  Check: 8173  Weight:  1.00

//
                    1                                                  50
hTRADEalpha_ MALKVLLEQE KTFFTLLVLL GYLSCKVTCE SGDCRQQEFR DRSGNCVPCN
  hTRAIN_1   MALKVLLEQE KTFFTLLVLL GYLSCKVTCE SGDCRQQEFR DRSGNCVPCN
hTRADEbeta_1 MALKVLLEQE KTFFTLLVLL GYLSCKVTCE TGDCRQQEFR DRSGNCVPCN
  hApo420pep MALKVLLEQE KTFFTLLVLL GYLSCKVTCE SGDCRQQEFR DRSGNCVPCN
   Consensus MALKVLLEQE KTFFTLLVLL GYLSCKVTCE SGDCRQQEFR DRSGNCVPCN

                   51                                                 100
hTRADEalpha_ QCGPGMELSK ECGFGYGEDA QCVTCRLHRF KEDWGFQKCK PCLDCAVVNR
  hTRAIN_1   QCGPGMELSK ECGFGYGEDA QCVTCRLHRF KEDWGFQKCK PCLDCAVVNR
hTRADEbeta_1 QCGPGMELSK ECGFGYGEDA QCVTCRLHRF KEDWGFQKCK PCLDCAVVNR
  hApo420pep QCGPGMELSK ECGFGYGEDA QCVACRLHRF KEDWGFQKCK PCLDCAVVNR
   Consensus QCGPGMELSK ECGFGYGEDA QCVTCRLHRF KEDWGFQKCK PCLDCAVVNR

                  101                                                 150
hTRADEalpha_ FQKANCSATS DAICGDCLPG FYRKTKLVGF QDMECVPCGD PPPPYEPHCA
  hTRAIN_1   FQKANCSATS DAICGDCLPG FYRKTKLVGF QDMECVPCGD PPPPYEPHCA
hTRADEbeta_1 FQKANCSATS DAICGDCLPG FYRKTKLVGF QDMECVPCGD PPPPYEPHCA
  hApo420pep FQKANCSATS DAICGDCLPG FYRKTKLVGF QDMECVPCGD PPPPYEPHCA
   Consensus FQKANCSATS DAICGDCLPG FYRKTKLVGF QDMECVPCGD PPPPYEPHCA

                  151                                                 200
hTRADEalpha_ SKVNLVKIAS TASSPRDTAL AAVICSALAT VLLALLILCV IYCKRQFMEK
  hTRAIN_1   SKVNLVKIAS TASSPRDTAL AAVICSALAT VLLALLILCV IYCKRQFMEK
hTRADEbeta_1 SKVNLVKIAS TASSPRDTAL AAVICSALAT VLLALLILCV IYCKRQFMEK
  hApo420pep SKVNLVKIAS TASSPRDTAL AAVICSALAT VLLALLILCV IYCKRQFMEK
   Consensus SKVNLVKIAS TASSPRDTAL AAVICSALAT VLLALLILCV IYCKRQFMEK

                  201                                                 250
hTRADEalpha_ KPSWSLRSQD IQYNGSELSC FDRPQLHEYA HRACCQCRRD SVQTCGPVRL
  hTRAIN_1   KPSWSLRSQD IQYNGSELSC FDRPQLHEYA HRACCQCRRD SVQTCGPVRL
hTRADEbeta_1 KPSWSLRSQD IQYNGSELSC LDRPQLHEYA HRACCQCRRD SVQTCGPVRL
  hApo420pep KPSWSLRSQD IQYNETELSC FDRPQLHEYA HRACCQCRRD SVQTCGPVRL
   Consensus KPSWSLRSQD IQYNGSELSC FDRPQLHEYA HRACCQCRRD SVQTCGPVRL

                  251                                                 300
hTRADEalpha_ LPSMCCEEAC SPNPATLGCG VHSAASLQAR NAGPAGEMVP TFFGSLTQSI
  hTRAIN_1   LPSMCCEEAC SPNPATLGCG VHSAASLQAR NAGPAGEMVP TFFGSLTQSI
hTRADEbeta_1 LPSMCCEEAC SPNPATLGCG VHSAASLQAR NAGPAGEMVP TFFGSLTQSI
  hApo420pep LPSMCCEEAC SPNPATLGCG VHSAASLQAR NAGPAGEMVP TFFGSLTQSI
   Consensus LPSMCCEEAC SPNPATLGCG VHSAASLQAR NAGPAGEMVP TFFGSLTQSI
```

Figure 1

```
                301                                                   350
hTRADEalpha_ CGEFSDAWPL MQNPMGGDNI SFCDSYPELT GEDIHSLNPE LESSTSLDSN
    hTRAIN_1 CGEFSDAWPL MQNPMGGDNI SFCDSYPELT GEDIHSLNPE LESSTSLDSN
hTRADEbeta_1 CGEFSDAWPL MQNPMGGDNI SFCDSYPELA GEDIHSLNPE LESSTSLDSN
  hApo420pep CGEFSDAWPL MQNPMGGDNI SFCDSYPELT GEDIHSLNPE LESSTSLDSN
   Consensus CGEFSDAWPL MQNPMGGDNI SFCDSYPELT GEDIHSLNPE LESSTSLDSN

                351                                                   400
hTRADEalpha_ SSQDLVGGAV PVQSHSENFT AATDLSRYNN TLVESASTQD ALTMRSQLDQ
    hTRAIN_1 SSQDLVGGAV PVQSHSENFT AATDLSRYNN TLVESASTQD ALTMRSQLDQ
hTRADEbeta_1 SSQDLVGGAV PVQSHSENFT AATDLSRYNN TLVESASTQD ALTMRSQLDQ
  hApo420pep SSQDLVGGAV PVQSHSENFT AATDLSRYNN TLVESASTQD ALTMRSQLDQ
   Consensus SSQDLVGGAV PVQSHSENFT AATDLSRYNN TLVESASTQD ALTMRSQLDQ

                401                   423
hTRADEalpha_ ESGAIIHPAT QTSLQEA~~~ ~~~
    hTRAIN_1 ESGAVIHPAT QTSLQEA~~~ ~~~
hTRADEbeta_1 ESGAIIHPAT QTSLQVRQRL GSL
  hApo420pep ESGAVIHPAT QTSLQVRQRL GSL
   Consensus ESGA-IHPAT QTSLQ--QRL GSL
```

09356034.030502

Figure 1

TRADE-α ESGAIIHPATQTSLQEA
TRADE-β ESGAIIHPATQTSLQVRQRLGSL
401
416
423

Figure 2

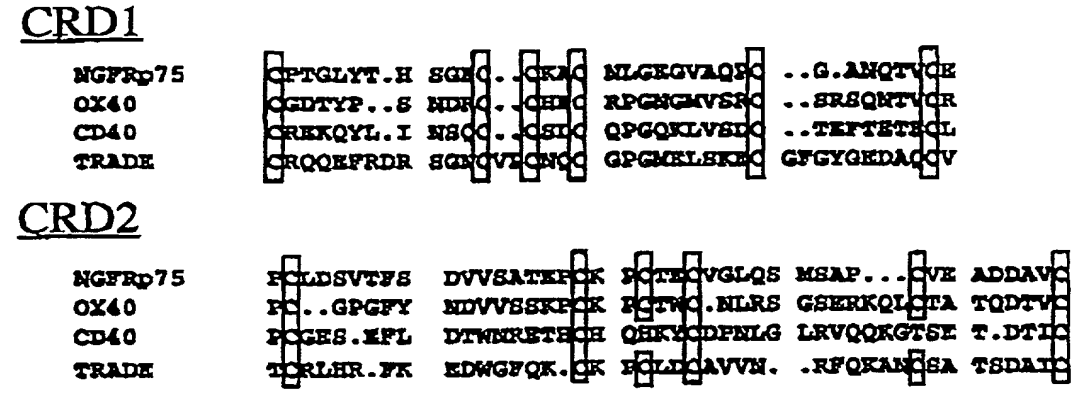


Figure 3

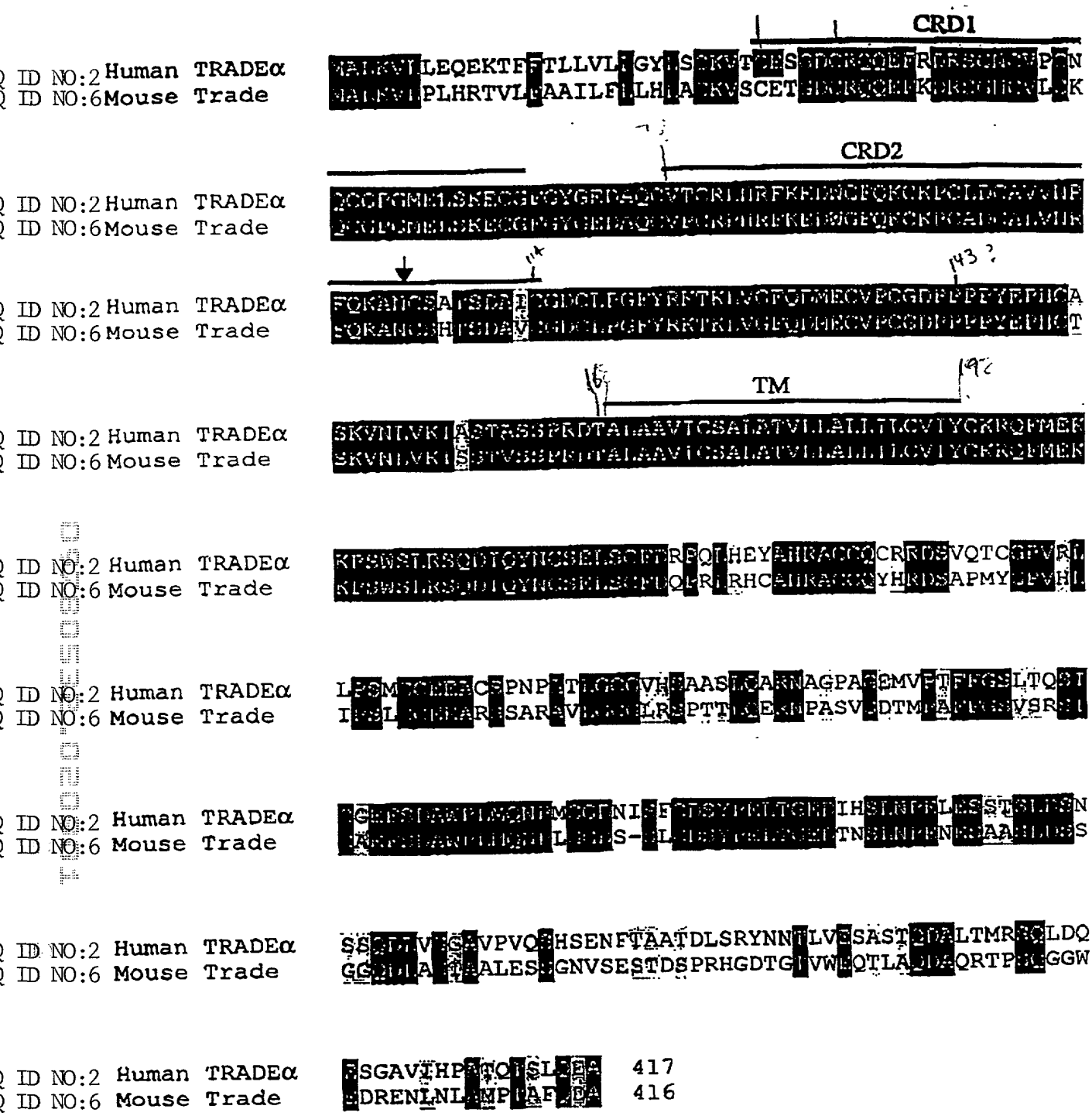


Figure 4

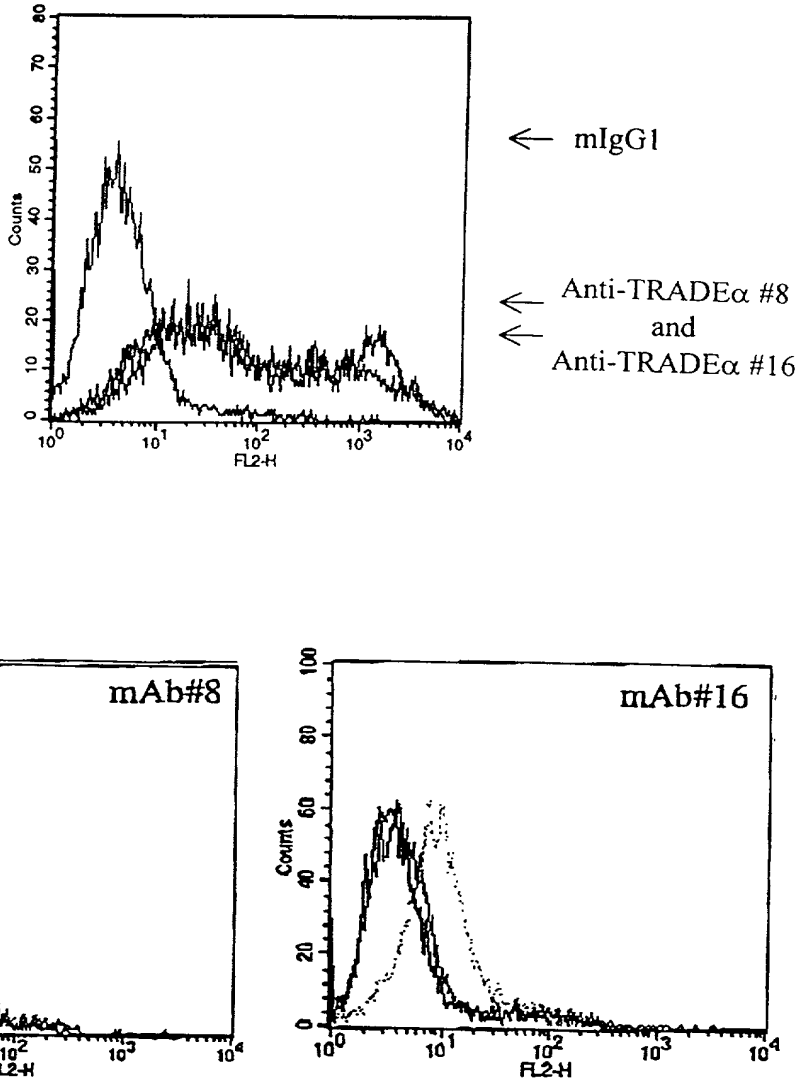
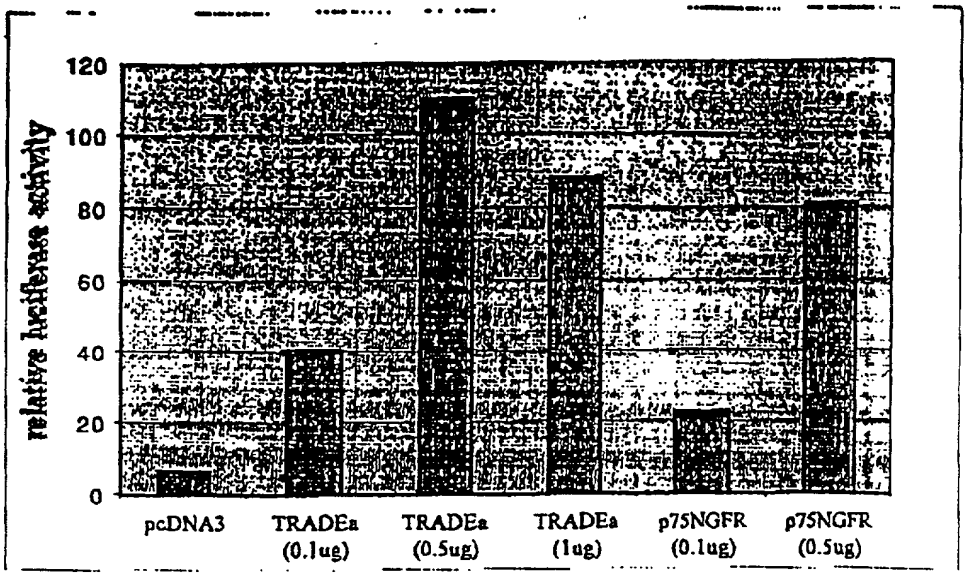
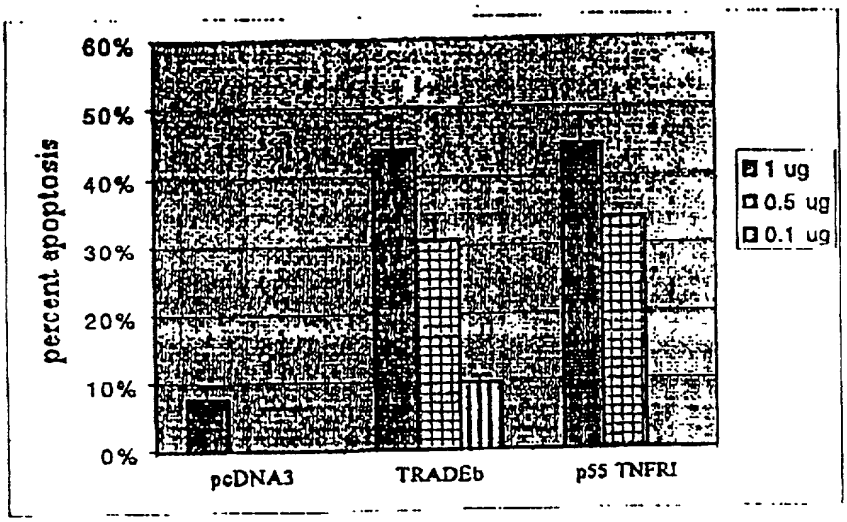

Counts
FL2-H
mIgG1
Anti-TRADEα #8
and
Anti-TRADEα #16
mAb#8
mAb#16
- mIgG1
- antibody
- antibody
+TRADE

Figure 5


relative luciferase activity
120
100
80
60
40
20
0
pcDNA3
TRADEa (0.1ug)
TRADEa (0.5ug)
TRADEa (1ug)
p75NGFR (0.1ug)
p75NGFR (0.5ug)

relative luciferase activity
16000
14000
12000
10000
8000
6000
4000
2000
0
pcDNA3
TRADEa (1ug)
TRADEa (0.5ug)
TRADEa (0.25ug)
TRADEa (0.1ug)
MEKK

Figure 6


60%
50%
40%
30%
20%
10%
0%
percent apoptosis
pcDNA3
TRADEb
p55 TNFRI
1 ug
0.5 ug
0.1 ug

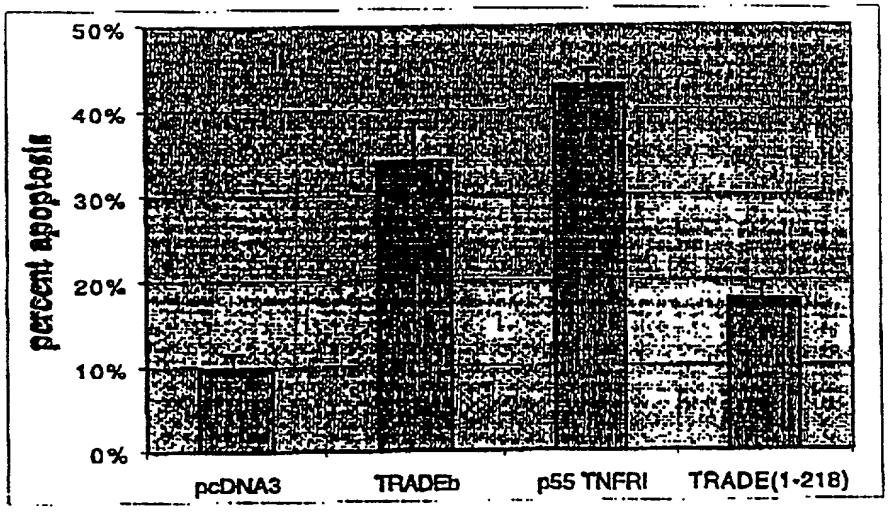

50%
40%
30%
20%
10%
0%
percent apoptosis
pcDNA3
TRADEb
p55 TNFRI
TRADE(1-218)

Figure 7

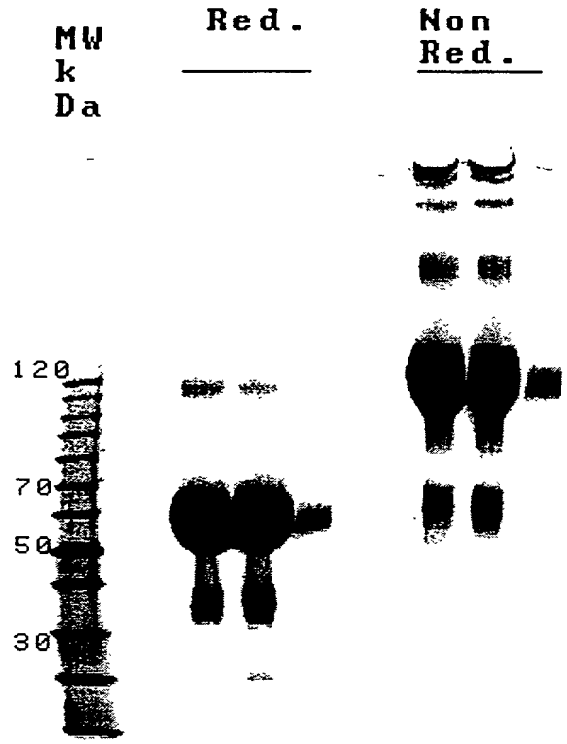
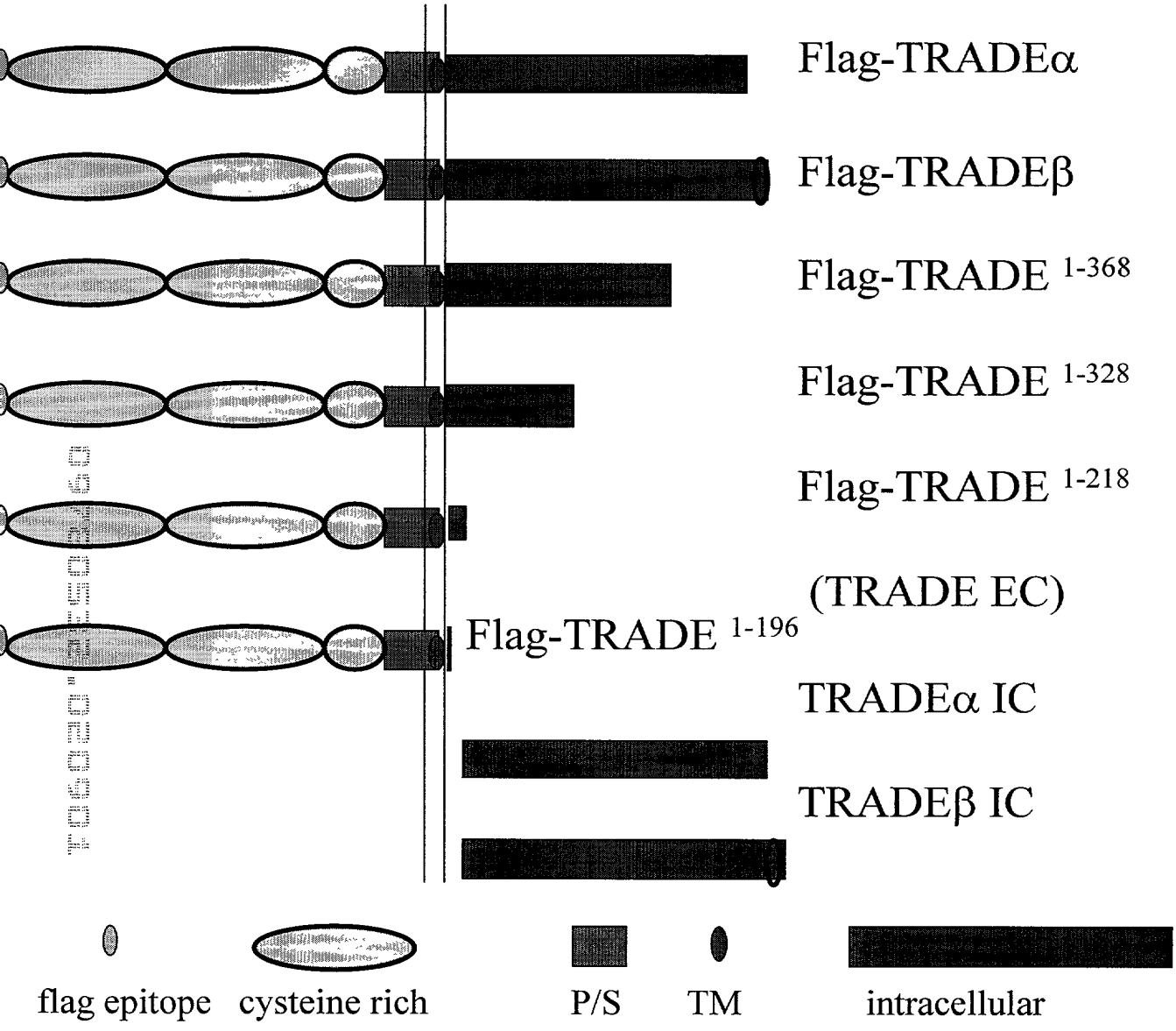


Figure 8



Deletion analysis

FIGURE 9

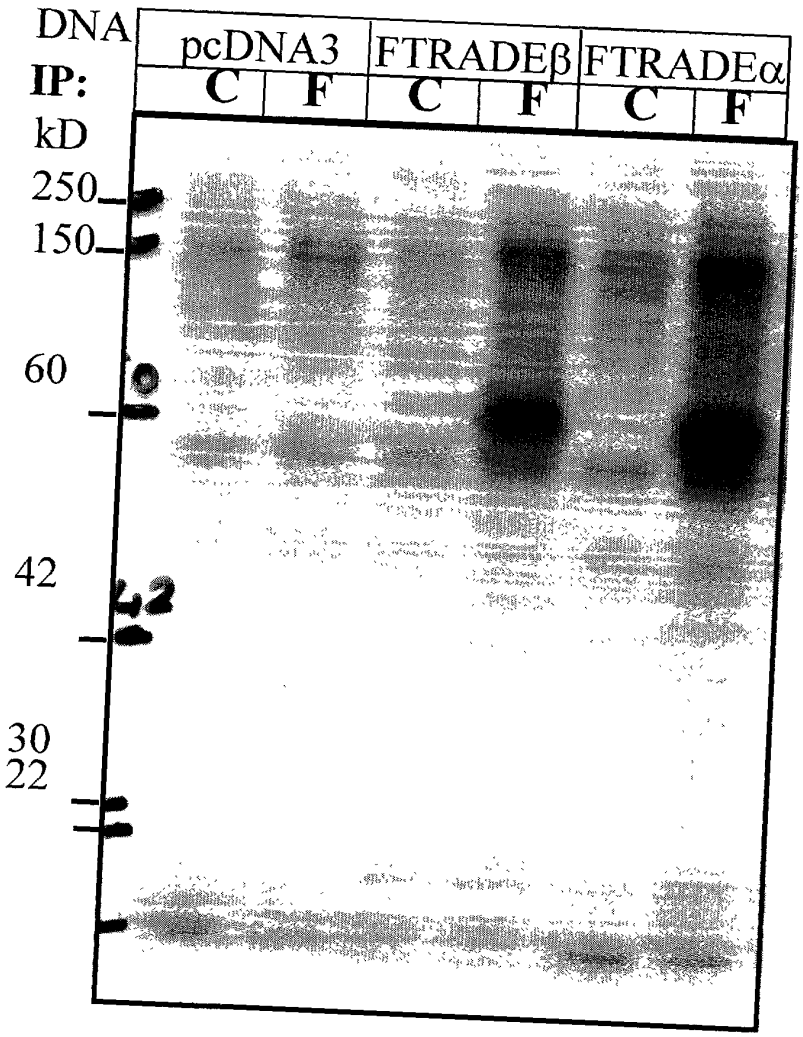
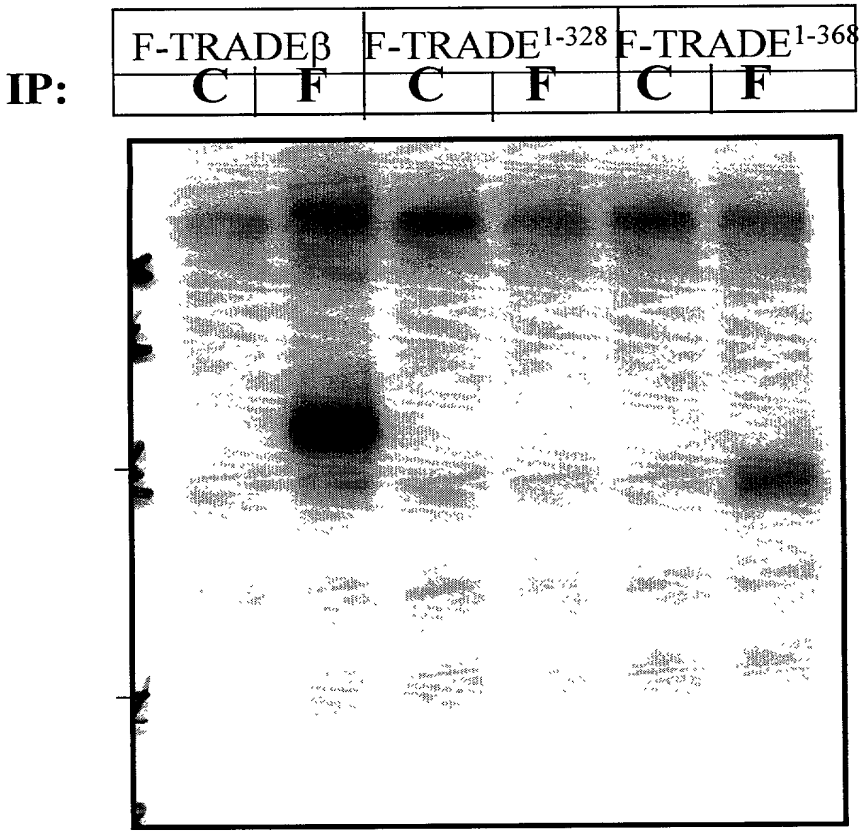


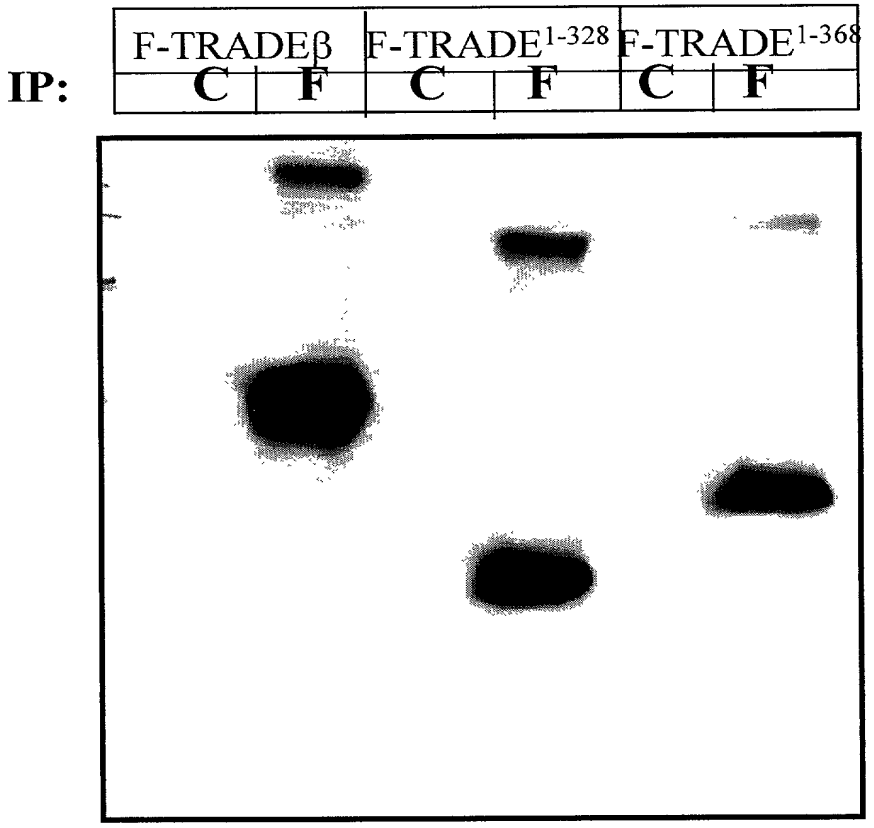
FIGURE 10

A



Kinase assay

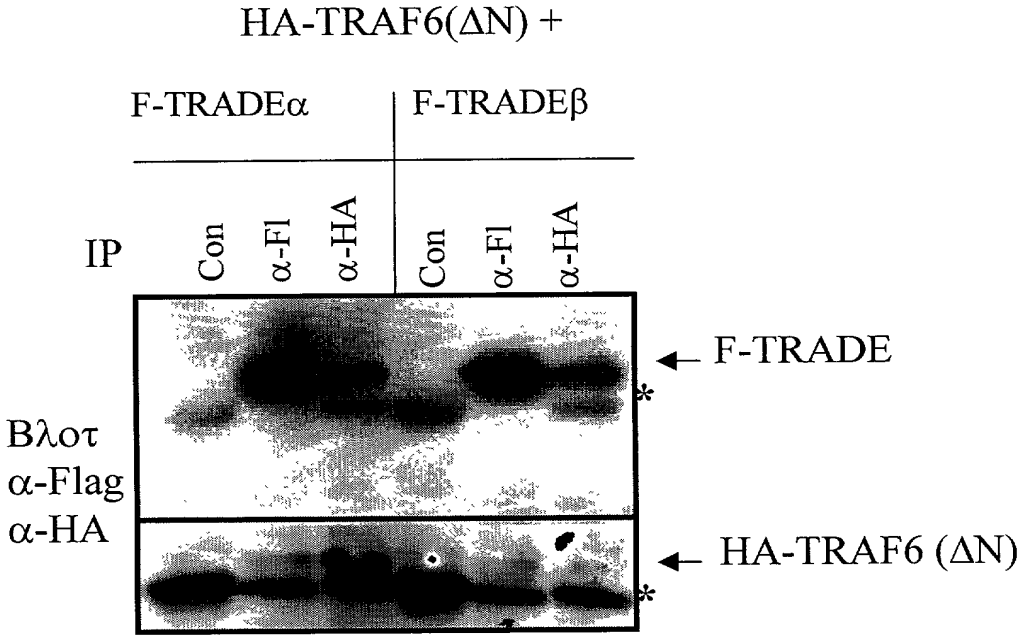
B



anti-Flag blot

FIGURE 11

A


HA-TRAF6(ΔN) +
F-TRADEα
F-TRADEβ
IP
Con
α-Fl
α-HA
Con
α-Fl
α-HA
Blot
α-Flag
α-HA
F-TRADE
HA-TRAF6 (ΔN)
*
*

B

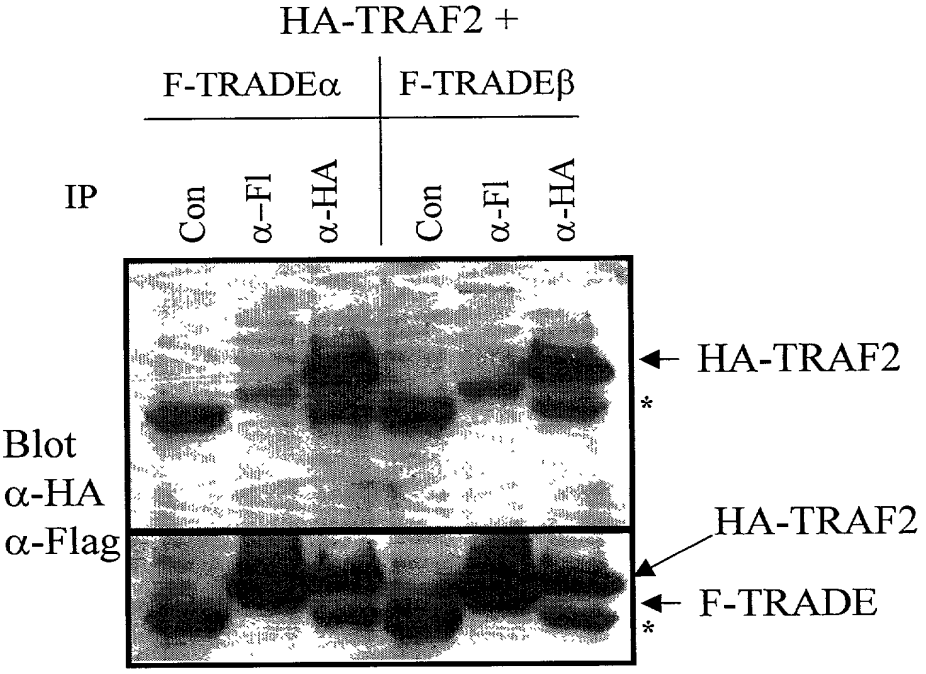

HA-TRAF2 +
F-TRADEα
F-TRADEβ
IP
Con
α-Fl
α-HA
Con
α-Fl
α-HA
Blot
α-HA
α-Flag
HA-TRAF2
HA-TRAF2
F-TRADE
*
*

FIGURE 12

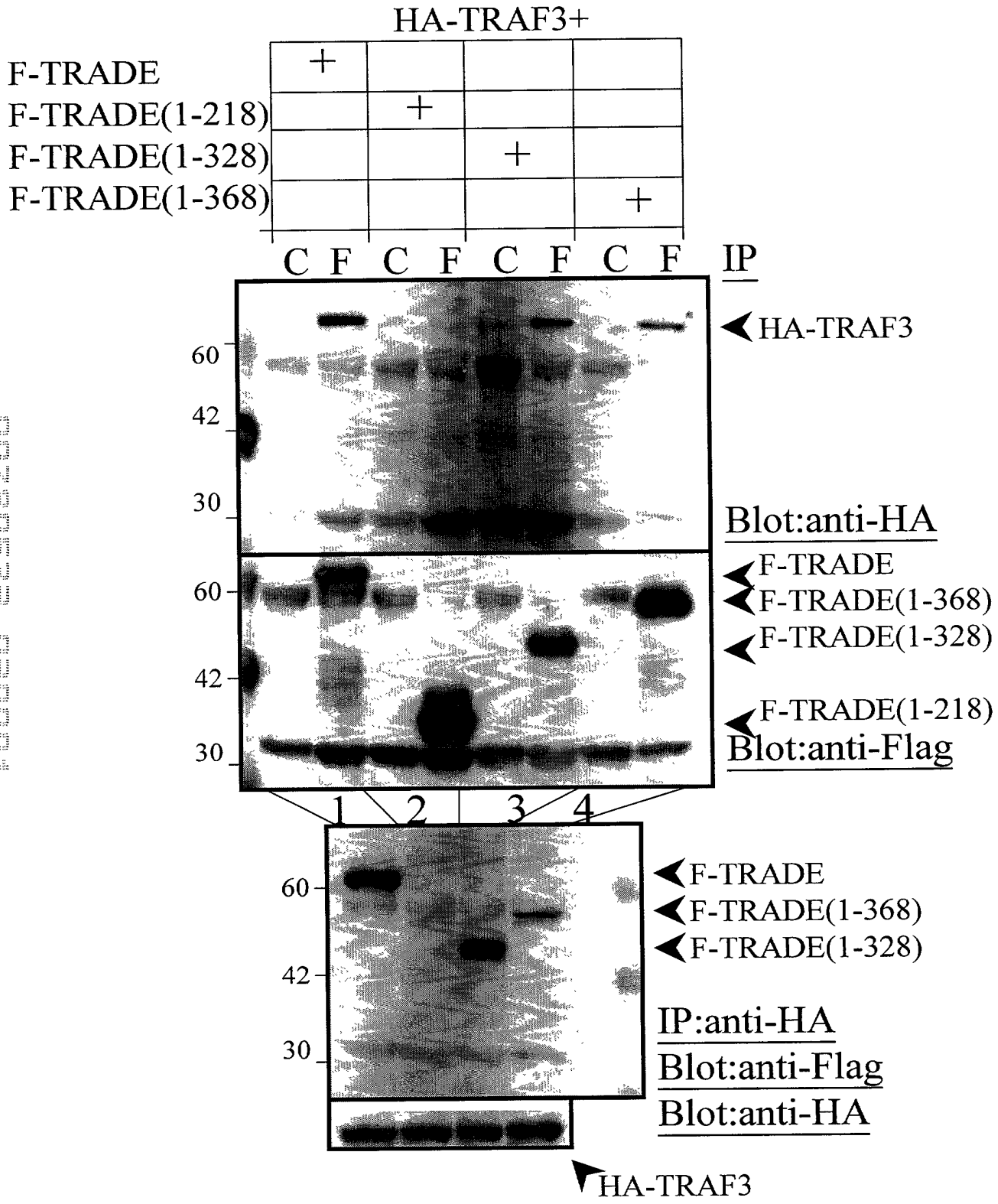

HA-TRAF3+
F-TRADE
F-TRADE(1-218)
F-TRADE(1-328)
F-TRADE(1-368)
C F C F C F C F
IP
HA-TRAF3
60
42
30
Blot:anti-HA
F-TRADE
F-TRADE(1-368)
F-TRADE(1-328)
F-TRADE(1-218)
Blot:anti-Flag
1 2 3 4
F-TRADE
F-TRADE(1-368)
F-TRADE(1-328)
IP:anti-HA
Blot:anti-Flag
Blot:anti-HA
HA-TRAF3

FIGURE 13

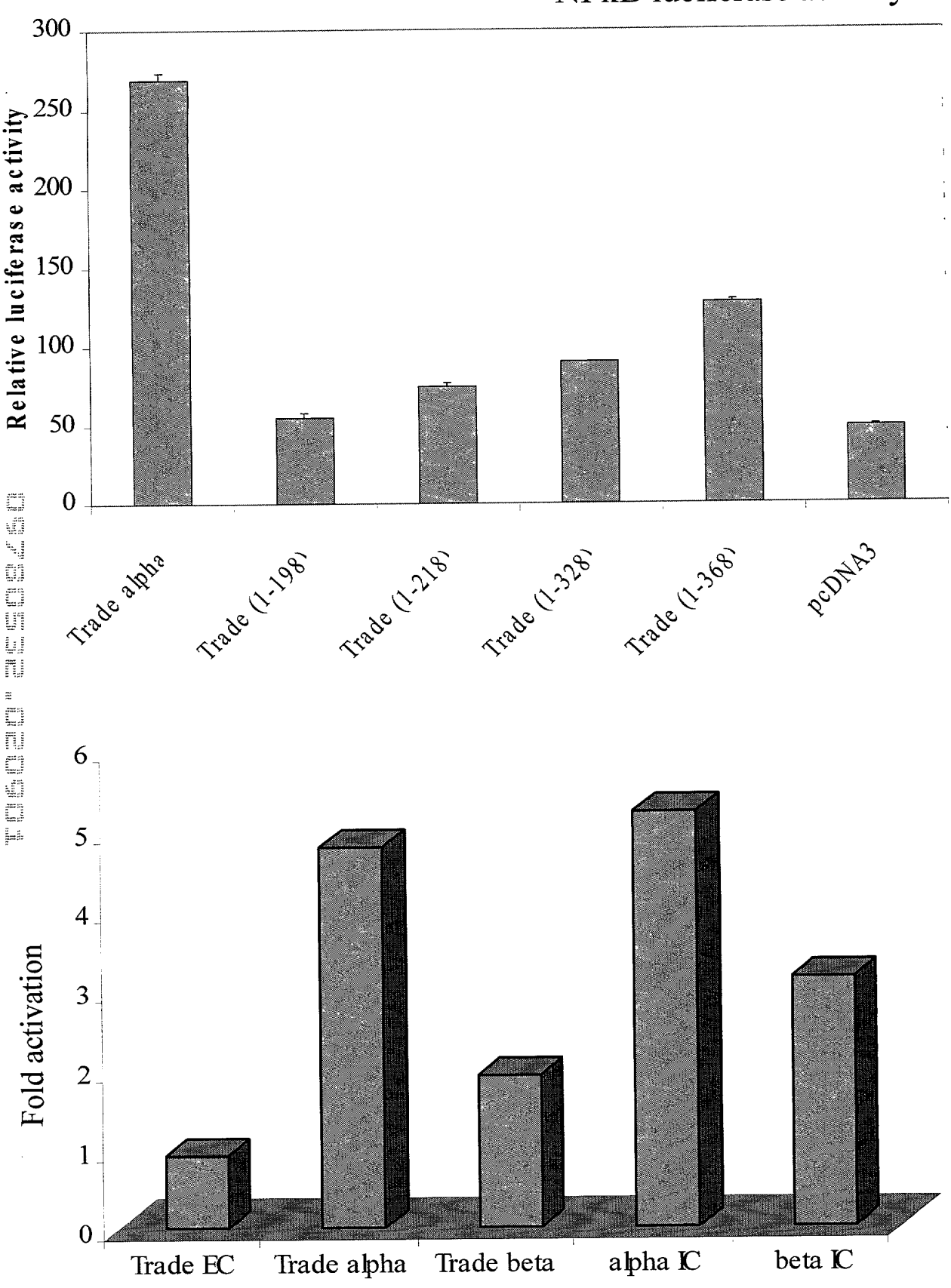

NFkB luciferase activity
Relative luciferase activity
300
250
200
150
100
50
0
Trade alpha
Trade (1-198)
Trade (1-218)
Trade (1-328)
Trade (1-368)
pcDNA3
Fold activation
6
5
4
3
2
1
0
Trade EC
Trade alpha
Trade beta
alpha IC
beta IC

FIGURE 14